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PEATLANDS

Land-Use Changes Associated with Oil Palm Plantations Impact PLFA Microbial Phenotypic Community Structure throughout the Depth of Tropical Peats

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Abstract

Tropical peatlands are complex and globally-important ecosystems that are increasingly threatened by anthropogenic disturbances, primarily via agricultural development. Microbes in peatlands play important roles in governing overall ecosystem functions and sustenance, with specific population dynamics governing carbon sink or source dynamics. We determined phenotypic microbial community structures under forest, drained, burned and oil palm plantation peatlands, using phospholipid fatty acid (PLFA) profiling. Communities were distinct under each land-use type, varied consistently with depth down to two metres, but with a distinct characteristic shift at circa one metre depth. There was bacterial dominance across all land-use types and depths. The burnt peatland showed the greatest contrast relative to forest, possibly due to the difference in water table level. Gram-positive bacteria was the most dominant group in surface layers under all land-use types except burnt, and their relative abundance decreased with depth, replaced by Gram-negative groups in deeper layers. Fungal relative abundance remained low across both land-use types and depth ranges. Our results shed light on a hitherto virtually unknown tropical peat microbial phenotypic community structure and indicate that anthropogenic disturbance in tropical peatlands severely alter microbial communities.

Keywords Burnt and drained peatlands · Land use change · Microbial community structure · Oil palm plantations · Tropical peatlands

Introduction

Tropical peatlands are complex and globally-important ecosystems supporting distinctive biodiversity and high carbon (C) storage capacity, related to their unique acidic, nutrient-poor and waterlogged environmental conditions (Parish et al. 2008; Miettinen et al. 2012). Microbes in peatlands play important roles not only in the formation of peat itself, but also directly influence ecosystem C turnover and nutrient mineralization required to support primary production, affecting overall ecosystem function and sustainability (Andersen et al. 2013). Different anthropogenic disturbances impact contrasting environmental and biological factors, affecting the overall balance of peatlands, and can turn the peatlands from C sinks to C sources (Page and Hooijer 2016; Hapsari et al. 2017)..

Oil palm expansion is one of the most extensive and ever-increasing anthropogenic disturbances faced by tropical peatlands in South East Asia (Miettinen et al. 2012; Rashid et al. 2013). During the conversion, forest land is usually

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completely cleared of vegetation to establish plantations (Bruhl and Eltz 2010; Luskin and Potts 2011). Oil palm plantations are generally maintained with regular harvesting, pruning, fertilization and pest management, causing significant changes to the environment and microclimate (Chung et al. 2000; Azhar et al. 2011; Luskin and Potts 2011). Observations from various studies in mineral soil systems show that oil palm plantations have relatively high soil and air temperature (Turner and Foster 2009; Lucey and Hill 2012), lower humidity (Turner et al. 2008; Gillespie et al. 2012), low pH similar to tropical forest (Chung et al. 2000) and very high temporal variation of such environmental characteristics (Gillespie et al. 2012; Lucey and Hill 2012). Documented environmental changes in tropical peatlands are limited and these studies showed similar patterns as seen in mineral soil (Tonks et al. 2017; Cooper et al. 2019; Dhandapani et al. 2019b, c). Though most mature oil palm plantations have a closed canopy (Peh et al. 2006), such canopies are typically homogenous and single-layered. This is unlike the multi-layered heterogeneous canopy structure characteristic of old-growth forests, which underpin a stable and moderate microclimate that provides a more appropriate habitat to provision understorey biodiversity. Another common feature is the high degree of soil disturbance arising from human movement and interference, and a complete lack of leaf-litter input in oil palm plantations (Peh et al. 2006; Cusack 2011).

Drained and burnt peatlands in South East Asia are either a pathway or by-product of oil palm expansion or logging (Bruhl and Eltz 2010). By 2015, 50% of the peatlands in Malaysia, Borneo and Sumatra were managed for agricultural plantations, and only 29% remain forested, of which most are selectively logged, with only 6% of the peat swamp forests in the region have no sign of disturbance since 1990 (Miettinen et al. 2016). The drainage of peatland reduces the level of the water table, consequently exposing the organic peat to aerobic decomposition mediated via microbes. The lowering of the water table can directly impact soil temperature, aeration, pH, and salinity (Coyne 1999; Turetsky et al. 2015). Responses to environmental change is typically more rapid for soil microbes than other flora and fauna because of their short generation times and higher population numbers (Andersen et al. 2013). It is widely demonstrated that soil microbes respond to environmental changes induced by anthropogenic land use changes (Bossio et al. 2005; Hartman et al. 2008; Kerfahi et al. 2014; Krashevska et al. 2015; Wood et al. 2017). A general inference from the observed pattern of the microbial responses is that soil chemical properties, especially pH, plays a key role in governing bacterial community structure (Hartman et al. 2008; Wood et al. 2017) while fungal community changes are more dependent on soil moisture level, C quality and composition (Thormann et al. 2004; Trinder et al. 2008, 2009; Asemaninejad et al. 2017).

Microbes in northern peatlands were found to vary with depth and be vertically stratified because of energy constraints such as organic matter quality, nutrient and oxygen availability, which become further influenced by disturbances (Sundh et al. 1997; Dedysh et al. 2006; Morales et al. 2006; Andersen et al. 2013). Some studies on northern peatlands found fungal communities to be dominant on surface peat layers (Newell et al. 1995; Kuehn et al. 2000) while other studies found bacterial communities to be dominant (Gilbert et al. 1998; Winsborough and Basiliko 2010). However for tropical forest, such studies are sparse. Hadi et al. (2001) measured greater bacterial than fungal biomass in both forest and paddy fields in Indonesian peatlands. In a rare study into microbial communities in Malaysian tropical peatlands, Jackson et al. (2009) found Acidobacteria to be the most dominant species in the bacterial community down to 50 cm depth, but did not study any eukaryotic soil communities. Similar trends of Acidobacteria dominance was also observed in forest, oil palm plantations and other disturbed peatlands in Indonesia (Mishra et al. 2014). Bacterial communities in Central American peatlands also had high proportion of Acidobacteria, but Proteobacteria were slightly higher in proportion than Acidobacteria (Troxler et al. 2012).

Too et al. (2018) observed significant change in microbial communities with depth in tropical peatlands, however the rhizosphere of different forest trees did not significantly influence change in microbial communities. Though the influence of vegetation on microbial communities is not yet clearly understood in the limited number of studies, the landscape level changes based on land-use in surface peat microbial community structure were evidently significant for different oil palm cropping systems and peat forests of different level of disturbances (Dhandapani et al. 2019a, b, c). Other than these mentioned studies, all the rest of the very few studies on microbes in tropical peatland focused on specific microbial functions such as activity and decomposition (Yule and Gomez 2009; Valenzuela et al. 2017), or on specific microbial groups (Voglmayr and Yule 2006; Hashidoko et al. 2008; Rahman et al. 2010; Arai et al. 2014). The lack of data and research on poorly understood peat microbial communities make it difficult to infer the structure and function of tropical peatland microbial communities and their response to disturbances in relation to land use change.

Analysis of ester-linked PLFA is an established method for phenotypic characterization of microbial communities (Kaur et al. 2005; Bloem et al. 2008; Frostegard et al. 2011). Phospholipids constitute 75% of the lipids in a cell membrane (Dowhan et al. 2016). The PLFAs respond to both intracellular and extracellular stress (Kaur et al. 2005), asserting the importance of PLFA analysis in monitoring soil microbes and their response to changes. A typical soil community phenotype profile obtained following PLFA analysis contains about 20 to 40 PLFAs (Bloem et al. 2008). Even though

PLFA analysis, unlike genetic-based molecular methods, does not give precise information on specific species composition, it is an effective, rapid and sensitive tool for monitoring microbial communities' responses to the environmental changes (Kaur et al. 2005; Bloem et al. 2008; Frostegard et al. 2011). A major limitation for using PLFA analysis in this study is that PLFA analysis does not detect methanogenic archaea, a functionally important group in peatlands. Methanogenic archaea contain ether linked fatty acids in their cell wall (Gattinger et al. 2003), while PLFA analyses extracts only ester-linked phospholipids.

The objectives of this research were to investigate and explore the variation of tropical peat microbial communities associated with (i) different land uses corresponding to different stages of oil palm expansion (ii) different depth within those land use types, and identify the dominant microbial community for each land use class and depth. Although microbial community structure were known to respond to changes in land-use and depth in many other ecosystems, tropical peatlands are distinct landscapes and microbial communities and responses are virtually unknown. We hypothesised that microbial community structures were affected and altered by the disturbances involved with different stages of conversion from forest to oil palm plantation, considering the dramatic changes in hydrology, microclimate and above ground biodiversity associated with the forest conversion. Similarly, microbial communities were hypothesised to change with depth due to the difference in carbon quality between surface layers and deeper layers along with changes in hydrology and oxygen availability with depth.

Materials and Methods

Study Sites

Study sites were selected in North Selangor peat swamp forest (NSPSF) in Peninsular Malaysia (3°34'32 N 101°15'44E) to represent land-use types involved in different stages of peatland conversion to oil palm plantations namely: (i) Historically drained secondary peat forest, hereafter denoted as 'Forest'; (ii) Recently drained peatland forest, denoted 'Drained'; (iii) Burnt peatland, denoted 'Burnt'; (iv) Recently cleared peatland with young oil palm plantation, denoted 'Young Oil Palm'; (v) Mature oil palm plantation, denoted 'Mature Oil Palm'.

North Selangor peat Swamp Forest (NSPSF) peatlands are about 10 km from the west coast of Malaysia (Yule and Gomez 2009). The Forest and Drained sites had similar above ground vegetation comprising *Macaranga pruinosa* (Miq.) Müll.Arg, *Campnosperma coriaceum* (Jack) Hallier f., *Blumeodendron tokbrai* (Blume) Kurz, *Shorea platycarpa* F.Heim, *Pararthocarpus venenosus* Becc., *Ixora grandiflora* Ker Gawl, *Pternandra galeata* Ridl., *Stenoclaena palustris* (Burm. f.) Bedd., *Asplenium longissimum* Baker, *Nephrolepsis biserrata* (Sw.) Schott, *Crytostachys sp.*, *Cyperus rotundus* L., and *Pandanus atrocarpus* Griff. (Yule and Gomez 2009). The major difference between the two forested land use types is the deep drainage in drained land use type with 2 to 3 m wide ditches dug at every few hundred metres (Tonks et al. 2017). The Forest sites in this study are chosen in areas that are away from old drainage ditches, and areas that had not been logged in the last 40 years resulting in high canopy cover and density than Drained sites. The site properties are given in Table 1. The mean values for peat properties and changes with depth are given in Supplementary Information S1. The Burnt sites have been subject to regular burning, with the most recent being 1.5–2 years before sampling, resulting in open scrubland, dominated by ferns, some grasses and bare soil and no forested areas. The burnt sites were also drain blocked, resulting in above surface water table in those sites (Table 1). The Young and Mature oil palm sites have undergone both drainage and deforestation with oil palm plants 9 months old, and 10–15 years old respectively.

Sampling was carried out during the wet season from November – December 2014, with five independent replicate sites for each land use type: precise locations are listed in Supplementary Table 1. Forest replicate sites were spatially distributed over the whole NSPSF. Mature Oil Palm sites were spatially distributed on the southern edge of NSPSF. Drained forest and Young Oil Palm were constrained to two areas on the north-eastern edge of NSPSF. Burnt sites were constrained to one area on the southern edge of NSPSF. A total of 5 replicate cores were collected from each of the 5 land-use type, bringing the combined total to 25 cores. Each core was 52 mm in diameter. The peat depth varied between sites and the core depth covered 2 m for all locations except Site 3 of the mature oil palm sites (viz. 100 cm, 50 cm and 150 cm for Sites 3, 4 and 5 respectively) and 2 forest sites (150 cm each for Sites 2 and 3). The peat cores were then separated into 20 cm segments and freeze-dried. The methods used for physico-chemical characterisation of the samples are given in Tonks et al. (2017). The physico-chemical characteristics measured by Tonks et al. (2017) were pH, peat temperature, peat moisture content, total carbon and nitrogen content, bulk density and organic matter content.

PLFA Extraction

Microbial community phenotypic structure was determined by phospholipid fatty acid (PLFA) analysis as described in Dhandapani et al. (2019a, b, c); PLFAs were extracted from 1 g freeze-dried tropical peat (from each 20 cm segment of each core) samples using a modification of the method described by Frostegard et al. (1991). The lipids from peat were

Table 1 GPS locations and environmental characteristics of sampling sites measured at the time of sampling (between 9 AM and 3 PM)

Site	GPS Coordinates	Surface moisture (%)	Surface temperature (°C)	Air temperature (°C)	water table level (cm)	peat depth (cm)
Forest 1	N03°41′42.9″ E101°11′04.7”	99.4	26	25.4	10	160
Forest 2	N03°29′02.4” E101°15′07.3”	93.3	26	28.5	−4	130
Forest 3	N03°41′49.7″ E101°11′08.5”	34	26.6	28.1	−27	80
Forest 4	N03°29′55.6” E101°12′01.5”	100	26	27	21	160
Forest 5	N03°39′08.2” E101°17′43.4”	100	25.8	27.6	30	415
Drained 1	N03°38′51.3″ E101°20′32.6”	36.1	27.8	29	−42	355
Drained 2	N03°38′04.2” E101°20′41.2	33	26.3	28.4	−8	241
Drained 3	N03°38′42.0” E101°20′35.0”	73.4	27.2	29.9	20	340
Drained 4	N03°39′30.7″ E101°19′19.9”	36.3	26	26.1	−10	200
Drained 5	N03°39′34.2” E101°19′24.7”	52.9	26.9	27.4	−30	200
Burnt 1	N03°27′56.5″ E101°26′29.08”	99.33	28.13	26.2	30	*
Burnt 2	N03°25′53.1” E101°20′12.6”	100	27.56	27.7	50	350
Burnt 3	N03°28′10.6″ E101°26′29.7”	100	27.63	32.2	17	350
Burnt 4	N03°27′45.3” E101°26′30.3”	100	28.93	29.6	20	350
Burnt 5	N03°25′56.2″ E101°20′14.0	100	27.23	27.4	40	265
Young OP 1	N03°39′49.8” E101°20′38.1”	39.02	29.63	26.6	−60	315
Young OP 2	N03°39′15.8” E101°20′28.3”	46.5	28.92	29.3	−26	200
Young OP 3	N03°39′17.8” E101°20′25.2”	52.45	29.4	32.2	−35	235
Young OP 4	N03°39′43.6” E101°19′42.5”	51.44	30.03	32	−25	254
Young OP 5	N03°39′40.2” E101°19′51.4”	38.57	29.49	33.3	−50	232
Mature OP 1	N03°24′43.6” E101°20′2.8”	44.59	27.99	28.8	−47	180
Mature OP 2	N03°25′26.3″ E101°20′05.3”	72.5	27.8	29.2	−18	225
Mature OP 3	N03°25′16.7″ E101°19′48.6”	85.8	29.14	28.1	−14.5	90
Mature OP 4	N03°27′55.8 E101°27′09.8”	63.12	27.55	25.6	−5	130
Mature OP 5	N03°24′49.9″ E101°20′10.0”	52.32	27.92	26.3	0	120

extracted using Bligh & Dyer extraction method (1959). The extracted lipids were then separated into neutral lipids, glycol lipids and polar lipids (containing phospholipids) fractions using Megabond Elut® silica gel column. The extracted polar lipids were then methylated by mild alkaline methanolysis and converted into fatty acid methyl esters, which were then run on gas chromatography.

Gas Chromatography and Peak Identification

The dried fatty acid methyl esters were suspended in 200 μl of hexane, ready for GC injection. One μl of each sample was injected into the GC in split-less mode. The column used in the GC for phospholipid analysis was ‘ZB-FFAP’ column, supplied by Phenomenex®. The column was 30 m length ×

0.25 mm inner diameter × 0.25 μm film thickness. The carrier gas was helium with the constant pressure of 18 psi. The initial oven temperature in GC was 120 °C; this was maintained for 1 min and then programmed to 250 °C at the rate of 5 °C min^{-1}. The constant temperature of 250 °C was maintained throughout the run. The results were displayed as a chromatogram of retention times of the compounds and the mass spectroscopy provides the ion profile of each compounds.

The fatty acids *i15:0, a15:0, i16:0, i17:0, a17:0* were considered as Gram-positive biomarkers (Wilkinson et al. 2002). The fatty acids *10me16:0* and *10me18:0* were described as the biomarkers for actinomycetes (Wilkinson et al. 2002; Moore-Kucera and Dick 2008), a group that belongs to Gram-positive bacteria. The relative abundance of Gram-negative bacteria was calculated using *16:1n9, 16:1n7, cyc17:0, 18:1n7* and *cyc19:0* as biomarkers (Wilkinson et al. 2002; Kaiser et al. 2010). *18:2n6* and *18:1n9* were used as fungal biomarkers (Vestal and White 1989; Wilkinson et al. 2002; Kaiser et al. 2010). Fatty acids such as *14:0, 16:0, 18:0, a17:1* and *20:0* were non-specific to any particular group (Wilkinson et al. 2002). The fatty acids with similar mass spectrum *18:1n9* and *18:1n7* were differentiated with the help of neutral lipid fatty acid (NLFA) analysis, by the findings that fungal biomarker *18:1n9* should have much higher NLFA/PLFA ratio than the Gram-negative biomarker *18:1n7* (Baath 2003). The ratio of Cyclopropane fatty acids (*cyc17:0& cyc19:0*) to their monoenoic precursors (*16:1n7 & 18:1n7*) and the ratio of total saturated fatty acids (*14:0, 16:0, 18:0, 20:0*) to mono-unsaturated fatty acids (*16:1n9, 16:1n7, a17:1n, 18:1n9, 18:1n7*) were used as indicators of stress (such as reduced carbon and nutrient availability) and other ecological conditions (such as flooding) (Bossio and Scow 1998).

Statistical Analysis

The statistical analyses were carried out using Genstat® 17th edition. Principal component (PC) analysis using correlation matrix was performed on PLFA data using Mol% normalised spectra. Relative abundance of individual microbial groups, and ratios between groups, were calculated and were subjected to statistical analysis using restricted maximum likelihood (REML) models, to identify the interactions of individual microbial groups with land use type, depth and combination of land use type and depth. Similar REMLs were also performed for PCs. REML was carried out by applying 'land use type' and 'depth' as a fixed factor, while 'core number' was applied as a random factor. Microbial groups were further analysed using Tukey's multiple comparison tests, to establish the significance of differences between each individual land-use types. Backward stepwise multiple regression was performed with relative abundance of microbial groups as response variables and other environmental parameters as fitted terms, to identify the relationship between microbial relative abundance and peat characteristics measured by Tonks et al. (2017). Simple linear regression was performed for microbial groups and ratios, and depths to identify their changes with depth.

Results

Principal component (PC) analysis showed significant discrimination for both land-use and depth. PC1–4 collectively accounted for 57% of the variation, with PC2–4 each accounting for similar proportions of variation between 10 and 13% (Fig. 1). There were no significant interactions between land-use type and depth for any of PC1–4 (Table 2) and hence main effects of land-use type and depth can be considered separately.

Effects of Land-Use Types

Across all depths, there was highly significant discrimination between land-use types across all of PC1–4 (Table 2; Fig. 1a-b). The primary nature of the discriminations were that PC1 separated Burnt from the other land-use types, and PC2 separated Forest from all the other land-use types. Young and Mature oil palm were strongly separated from each other by PC3, with the latter being distinct from all the other land-use types. Burnt land-use were intermediate to the others with respect to PC3. PC4 then separated Mature and Young Oil Palm from all others, which otherwise were clustered with respect to this component. The loadings for individual PLFAs associated with each of the PCs were generally dispersed, with no particular dominance of any single PLFA, and *20:0*, *18:2n6* and *16:0* having very low loading scores (Fig. 1c-d). Notable exceptions were that *16:0* was particularly associated with the separation of Forest from all the other land-use types with respect to PC1 and 2 (Fig. 1a, c). Likewise, *14:0*, *10me18*, *17:1n* and *i17:0* were strongly associated with the distinctness of both Mature and Young Oil Palm land-uses with respect to PC4 (Fig. 1b, d).

Variations along the Depth

Across all treatments, PC1 and PC3 (collectively accounting for 35% of the variation; Fig. 2), varied significantly with respect to depth (Table 2). Both PCs showed a consistent and coherent relationship to depth, resulting in a distinct trajectory in the biplot (Fig. 2a). There was a distinct shift in this trajectory at around 1 m depth, below which PC2 showed little change (Fig. 2a). The loadings for individual PLFAs associated with each of the PCs were generally dispersed, with no particular dominance of any single PLFA and *18:2n* and *14:0* having very low loading scores (Fig. 2b).

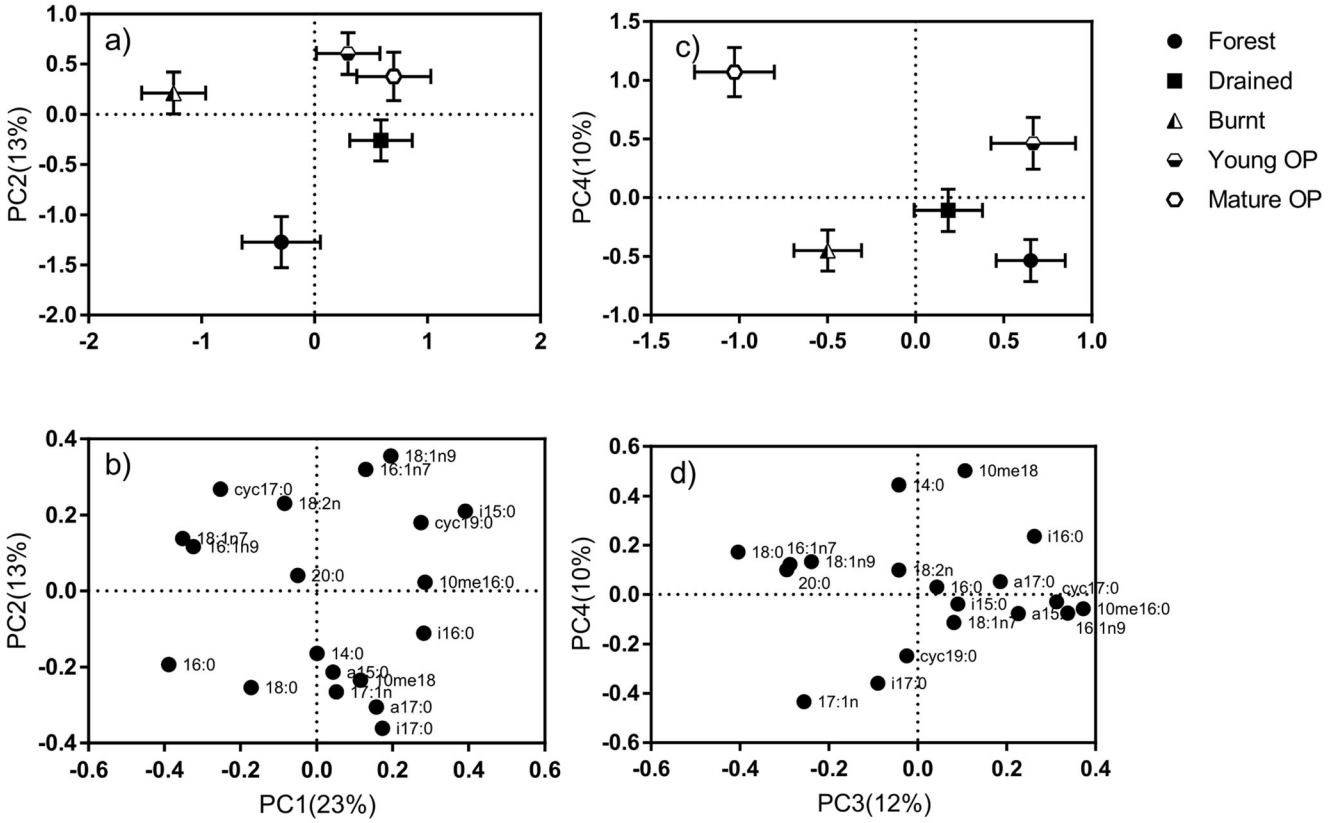

Fig. 1 Effect of land-use type (OP = oil palm) upon phenotypic structure of soil microbial communities determined by PLFA analysis, as shown by principal component (PC) analysis. **a**, **b** ordination of PC1 and 2 and associated loadings for individual PLFAs; **c**, **d** ordination of PC3 and PC4 and associated loadings, point labels denote PLFA type. For **a**, **c** points denote means (n varies, see text), whiskers denote standard errors

Relative Abundance and Microbial Community Structure

Only the proportion of Gram-negative bacteria, and fungi varied significantly between land-use type, with a significant interaction between land-use type and depth for fungi (Table 3). The statistical difference between the land-use types for fungal relative abundance was mainly driven by Forest, as here fungal relative abundance reduced with depth, while for all the other land-use types, the proportion of fungal markers was maintained across all depths (Fig. 3). Further Tukey's multiple comparison tests showed that Gram-negative relative abundance was significantly different in Burnt land-use from all the rest of the land use types (Supplementary information S2). Mature Oil Palm had significantly different fungal relative abundance compared to Forest. Forest land-use was different from Young Oil Palm land-use in both Gram-negative and fungal relative abundance. Burnt land use had significantly different Gram-positive relative abundance to that of Drained and Forest land-use types. Actinomycetes did not significantly differ among any land-use types, and non-specific PLFAs differed only between Forest and Young Oil Palm. The ratio between Gram-negative and Gram-positive bacteria (G-:G+) differed significantly between Burnt land-use and all the rest of the land-use types except Mature Oil Palm. Mature

Table 2 Linear mixed model (REML) for principal component scores (PC1–4) obtained from aggregated PLFA data with respect to microbial groups, showing statistical significance of the effects of land-use type, depth and the interactions between land-use type and depth

REML	PC1			PC2			PC3			PC4		
	df	F ratio	p	df	F ratio	P	df	F ratio	p	df	F ratio	p
Land-use type	4, 165	14.6	<0.001	4, 165	8.59	<0.001	4, 165	12.57	<0.001	4, 165	12.29	<0.001
Depth	9, 165	23.6	<0.001	9, 165	0.69	0.721	9, 165	2.87	0.004	9, 165	0.65	0.752
Land-use type*Depth	36, 165	0.77	0.817	36, 165	0.48	0.994	36, 165	0.92	0.608	36, 165	1.42	0.072

The 'p' values below '0.05' represent statistically significant effect

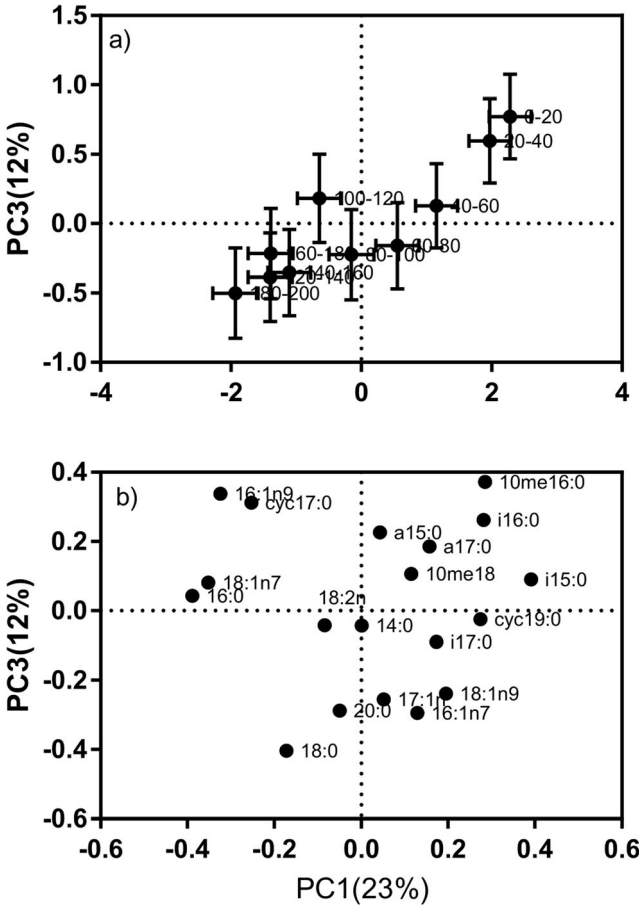

Fig. 2 Relationship between soil depth and phenotypic structure of soil microbial communities determined by PLFA analysis, as shown by principal component (PC) analysis. **a** ordination PC1 and PC3; points denote means across all land-use types (n varies, see text) (OP = oil palm), whiskers denote standard errors. Point labels denote depth of sample in cm from surface; **b** associated loadings for individual PLFAs, point labels denote PLFA type

Oil Palm land-use had significantly different Fungi:bacteria (F:B) ratio to that of Burnt and Forest land-use types. The F:B ratio was also significantly different between Young Oil Palm and Forest land-use types. None of the microbial groups or ratios significantly differed among Drained, Young Oil Palm and Mature Oil Palm land-use types (Supplementary information S2).

All the microbial groups showed significant changes with depth (Table 3). All of the land-use types were dominated by bacteria at all depths. Gram-positive bacteria (including the Gram-positive subgroup, actinomycetes) was the dominant group at the surface peat for all land-use types except for the Burnt peatland, where Gram-negative bacteria was the most dominant group at the surface by a large margin (Fig. 3). The proportion of Gram-positive bacteria decreased (= 25.343–0.07974*depth cm; $F_{(1,214)} = 126.45$, $p < 0.001$, $R^2 = 0.37$) and that of non-specific fatty acids increased (= 22.6 + 0.0941*depth cm; $F_{(1,214)} = 84.94$, $p < 0.001$, $R^2 = 0.28$) with depth across all land uses. This decrease in proportion of Gram-positive biomarkers made way for the Gram-negative bacterial group (=36.98 + 0.0208*depth cm; $F_{(1,214)} = 3.94$, $p = 0.049$, $R^2 = 0.014$) to be the dominant one with increasing depth, starting at 40–60 cm for Forest and 20–40 cm for all the other land-use types (Fig. 3). The relative abundance of fungal markers was less than 10% and slightly decreased (=7.307–0.010*depth cm; $F_{(1,214)} = 7.08$, $p = 0.008$, $R^2 = 0.028$) maintaining a similar lower level across all depths, irrespective of the land-use types. The proportions of actinomycetes were also less than 10% across all land-use types, and followed a similar trend of decrease with depth (=7.766–0.026*depth cm; $F_{(1,214)} = 49.63$, $p < 0.001$, $R^2 = 0.185$) as other Gram-positive bacteria.

In general, the ratio between fungal and bacterial relative abundance (F:B) did not vary significantly with respect to depth ($F_{(1,214)} = 0.07$, $p = 0.799$; Fig. 4a). The ratio of Gram-negative to Gram-positive bacteria (G-:G+) generally increased with depth (=0.863 + 0.015*depth cm; $F_{(1,214)} = 39.53$, $p < 0.001$, $R^2 = 0.153$) for all land-use types, but with different patterns of behaviour according to land-use type. Burnt land-use involved a rapid increase in ratio (G-:G+) down to 140 cm depth and decline thereafter, with a similar trend for Forest although more attenuated (Fig. 4b). Cleared, Mature and Young Oil Palm land-use showed a distinct G-:G+ ratio increase with depth commencing at 160 cm (Fig. 4b).

Table 3 Linear mixed model (REML) for microbial group relative abundances, showing statistical significance of the effects of land-use type, depth and the interaction between land-use type and depth

REML	G+			Act			G-			Fungi			Non-specific		
	df	F ratio	p	df	F ratio	p	df	F ratio	p	df	F ratio	p	df	F ratio	p
Land-use type	4, 19.6	1.61	0.211	4, 18	1.43	0.265	4, 19.6	4.11	0.014	4, 19.8	3.87	0.018	4, 17.4	2.65	0.069
Depth	9, 147	19	<0.001	9, 146	7.47	<0.001	9, 147	3.31	0.001	9, 148	2.29	0.02	9, 145	13.3	<0.001
Land-use type*Depth	36, 147	1.23	0.198	36, 147	1.23	0.194	36, 147	0.17	0.168	36, 148	1.65	0.021	36, 146	0.97	0.531

The 'p' values below '0.05' represent statistically significant effect.Gram-positive (G+) represents the sum of relative abundance of PLFAs *i15:0, a15:0, i16:0, i17:0* and *a17:0.* Actinomycetes (Act) represents the sum of *10me16:0* and *10me18:0.* Gram-negative (G-) represents the sum of *16:1n9, 16:1n7, cyc17:0, 18:1n7* and *cyc19:0.* Fungi represents sum of *18:2n6* and *18:1n9.* Non-specific represents the sum of *14:0, 16:0, 18:0, a17:1* and *20:0*

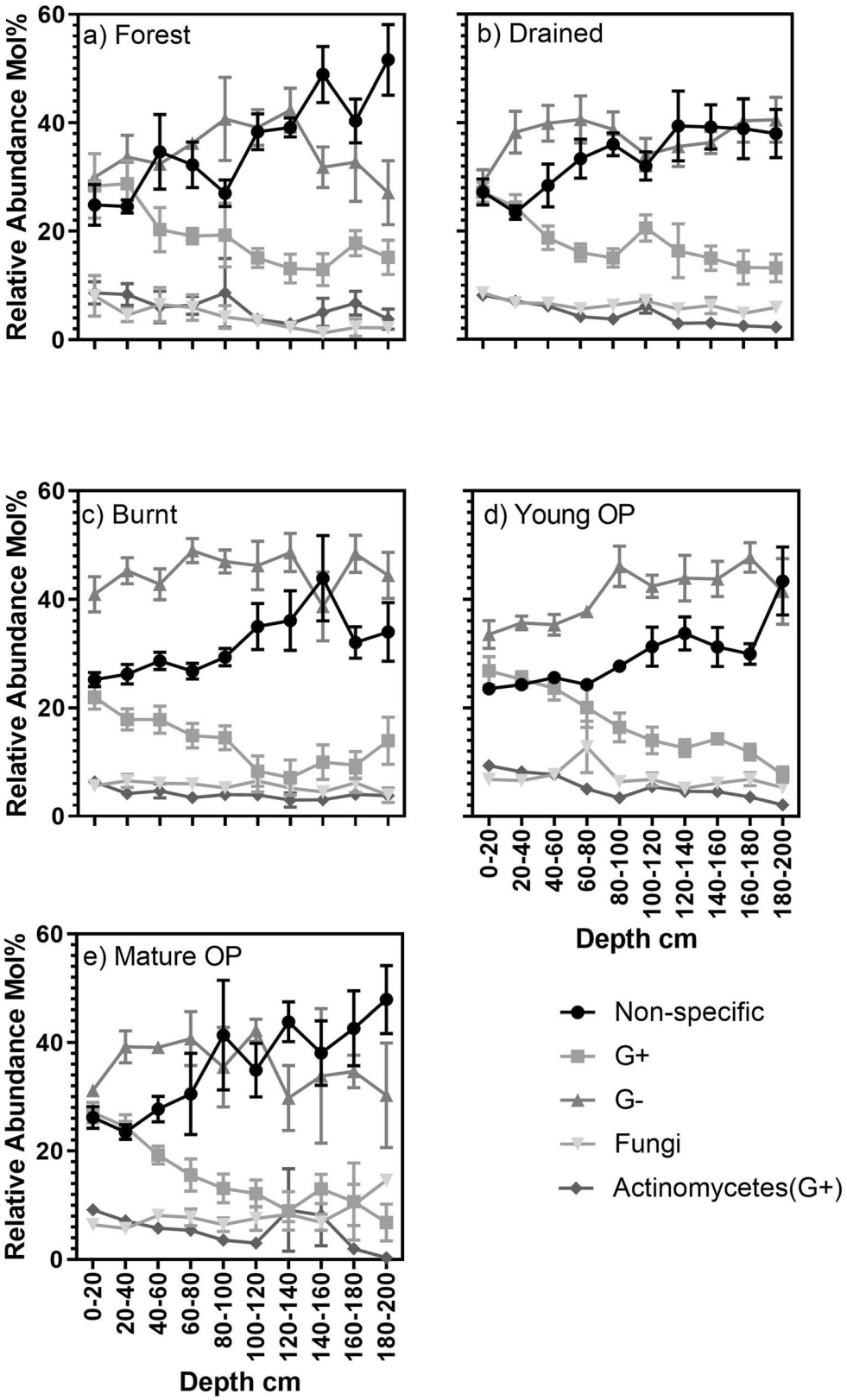

Fig. 3 Relative abundance of different microbial groups as determined by PLFA analysis in relation to soil depth ranges under **a** forested peatland; **b** drained peatland; **c** burnt peatland; **d** young oil palm (OP) plantation; **e** mature oil palm (OP) plantation. Points denote means (n varies, see text), whiskers denote standard errors

Principal component analysis using relative abundance of microbial groups strongly supported the variations between land-use types observed via principal component analysis of individual fatty acids (Supplementary information S3). PC1 and PC2 together accounted for 73% of the variations. PC1 separated out Burnt from all of the other land-use types, while

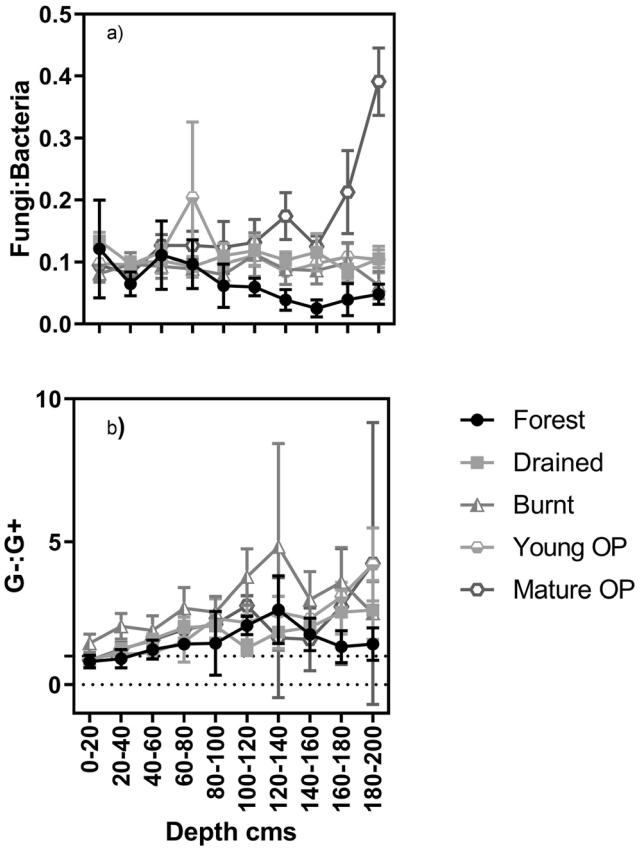

Fig. 4 Ratios of relative abundance of microbial groups across soil depth range under different land-use types (OP = oil palm). **a** ratio between the relative abundance of fungi and bacteria; **b** ratio between the relative abundance of Gram-negative bacteria and Gram-positive bacteria. Points denote means (n varies, see text), whiskers denote standard errors

PC2 separated Forest from the other land use types. Burnt land-use was separated farthest away from Forest by both the PCs, the changes with depth and a shift in pattern of change after a metre depth was also apparent in microbial group level PCs (Supplementary information S3).

Other Ecological Indicators

The ratio between cyclopropane fatty acids and their precursors (cyc:pre) decreased with depth for all the reported land-use types ($F_{9,144} = 15.75$, $p < 0.001$), while the difference among land-use types, and interaction between land-use type and depth was not significant (Fig. 5). Burnt land-use had the lowest cyc:pre ratio in the surface peat layer and the changes with depth were minimal in Burnt compared to other land-use types. Simple linear regression showed a significant decrease of cyc:pre ratio with depth (=2.878–0.017*depth cm; $F_{(1,214)} = 81.45$, $p < 0.001$, $R^2 = 0.273$) for all land use types.

The ratio of total saturated fatty acids to mono-unsaturated fatty acids (sat:mono) varied with depth ($F_{9,140} = 1.98$, $p < 0.05$) and land-use types ($F_{4,\ 15} = 3.76$, $p < 0.005$), but the interaction between land-use type and depth was insignificant. Forest, Drained and Mature Oil Palm had identical ratio at the surface, while Burnt and Young Oil Palm had identical lower ratio at the surface layer. The ratio increased for Forest and it either decreased or remained the same with depth for other land-use types. The Mature and Young Oil Palm land-use types had similar higher ratios at and after a metre depth. Burnt land-use type had the lowest sat:mono ratios across all depth ranges except for 140–160 cm. Simple linear regression could not identify significant change in sat:mono ratio with depth ($F_{(1,214)} = 2.57$, $p = 0.110$).

Microbial PLFA Relative Abundance and Environmental Controls

Overall, volumetric moisture was a significant predictor for most microbial groups and PLFA ratios (Figs. 6 and 7). Gram-positive bacteria and fungi responded negatively, while Gram-negative bacteria responded positively to increase in volumetric moisture. Among ratios, G-:G+ responded positively and cyc:pre responded negatively to moisture. Other than the

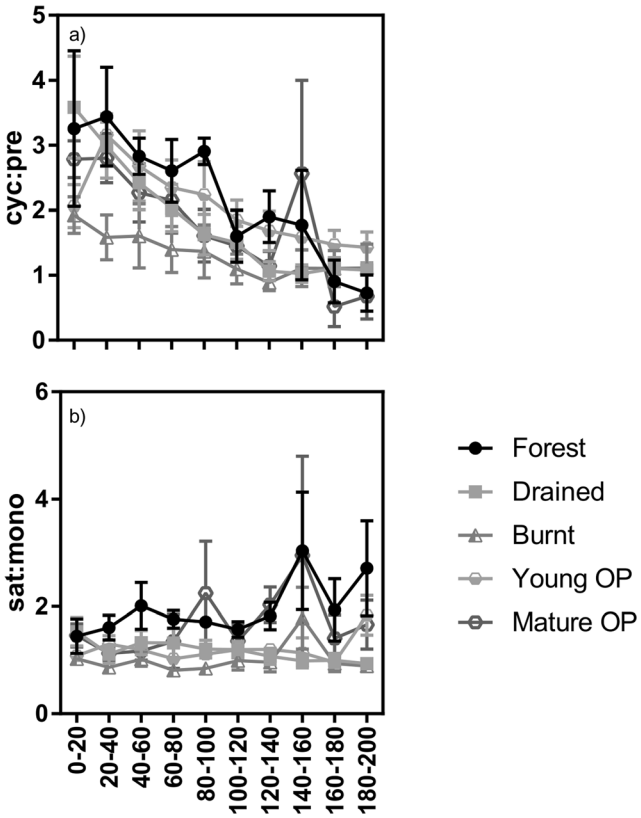

Fig. 5 PLFA ratios used as stress indicators as determined by PLFA analysis in relation to soil depth ranges under different land-use types (OP = oil palm). **a** ratio between cyclopropance fatty acids and their precursors; **b** ratio between saturated fatty acids and mono-unsaturated fatty acids. See text for further details regarding derivation and interpretation. Points denote means (n varies, see text), whiskers denote standard errors

responses to volumetric moisture, actinomycetes positively responded to bulk density (Fig. 6a) and negatively to C:N (Fig. 6b), Gram-positive bacteria responded positively to bulk density, and fungi responded positively to organic matter content. Relative abundance of non-specific fatty acids and F:B responded positively to C:N and organic matter content respectively. Cyc:pre and Sat:mono were negatively correlated with C:N and organic matter content respectively.

Discussion

Microbes under Different Land-Use Types

This research compared sites at different stages of oil palm conversion from forest, including draining of forest, followed by clearing, or burning in some cases, and establishing oil palm plantations. Microbial phenotypic community structure

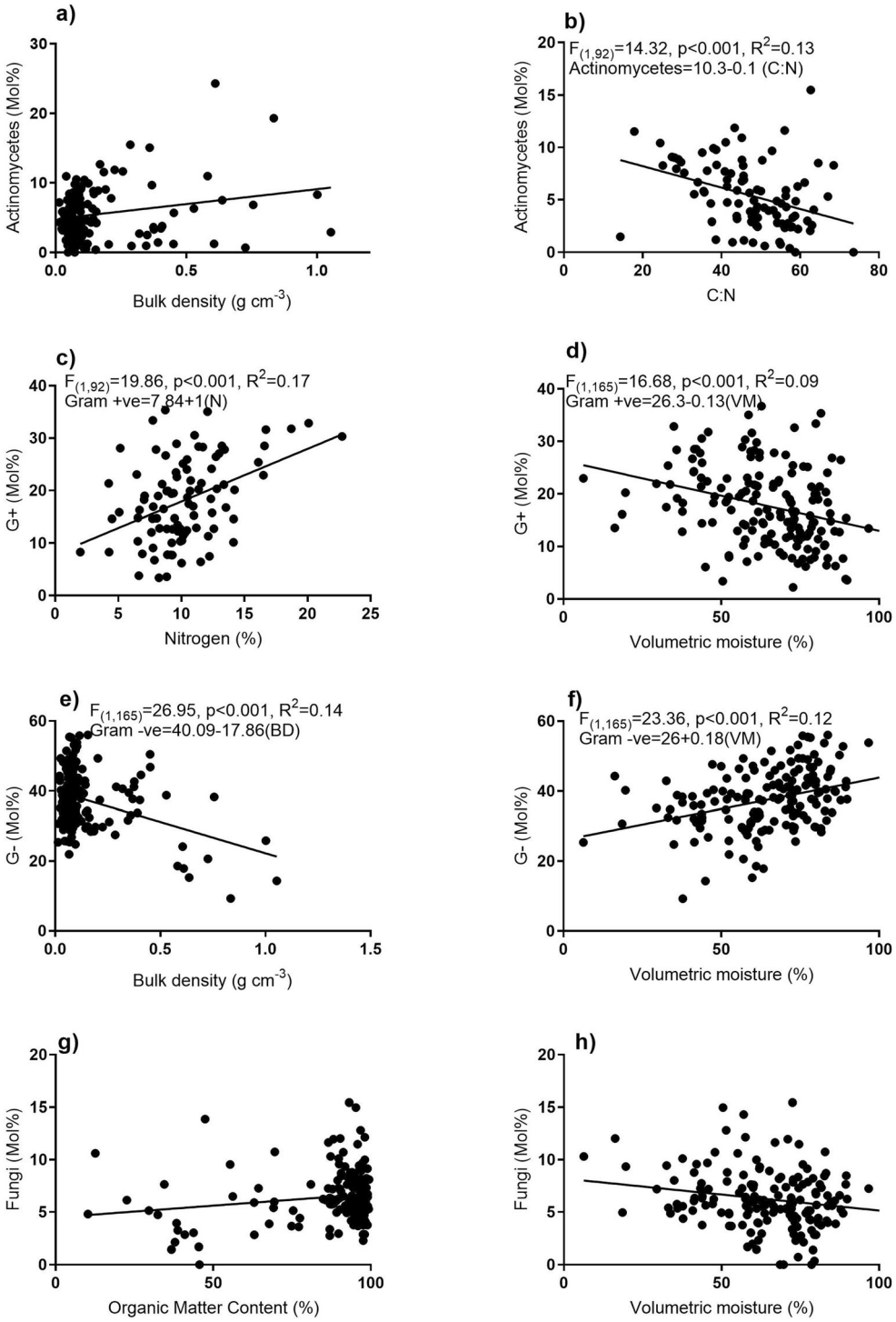

Fig. 6 Relationship between **a** Actinomycetes and bulk density **b** Actinomycetes and Carbon:Nitrogen ratio **c** Gram-positive bacteria and Nitrogen **d** Gram-positive bacteria and volumetric moisture **e** Gram-negative bacteria and bulk density **f** Gram-negative bacteria and volumetric moisture **g** Fungi and organic matter content **h** Fungi and volumetric moisture. The regression equation is not shown for **a**, **g** and **h** as the models were poor fit with R^2 lower than 0.05

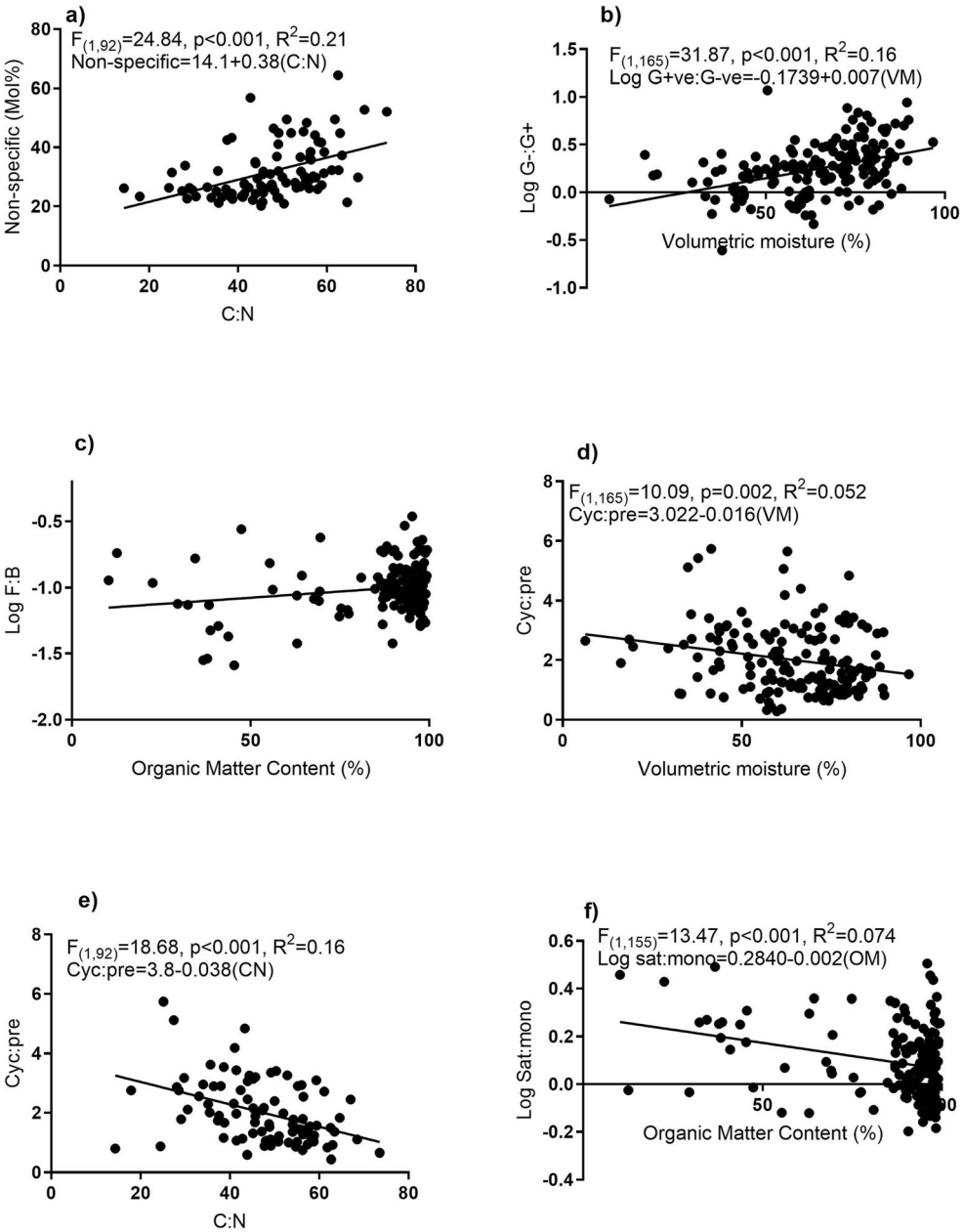

Fig. 7 Relationship between **a** Non-specific fatty acids and Carbon:Nitrogen ratio **b** Log Gram-negative:Gram-positive ratio and volumetric moisture **c** Log fungi:bacteria ratio and organic matter content **d** Cycyclopropane:precursors fatty acid ratio and volumetric moisture **e** Cyclopropane:precursors fatty acid ratio and carbon:nitrogen ratio **f** Log Saturated:monounsaturated fatty acid ratio and organic matter content. The regression equation is not shown for **c**, as the model was a poor fit with R^2 lower than 0.05

throughout the depth of 2 m in tropical peatlands are distinct for each land-use type. These five types of land-use along with other managed production forest, cover most of South East Asian peatlands (Miettinen et al. 2012; Miettinen et al. 2016). Given the environmental changes that accompany these land uses and changes in physico-chemical properties at different stages of conversion (Tonks et al. 2017), it is not surprising to see that they have significantly different microbial phenotypic community structure. As expected, Forest had notably distinct microbial communities, while the Drained peatlands had microbial communities that was similar to those in oil palm plantations. The Young Oil Palm plantation site in this study was a recently converted forest land, cleared without the use of fire. It is not uncommon to use fire to clear sites for oil palm plantation or use/claim fire affected sites for plantations, even though legislation in both Indonesia and Malaysia prohibits it (Varkkey 2013, 2016). It is highly likely that oil palm plantations established on fire affected site would have very different microbial community structure to that of oil palm plantation in this study, as our results have shown that oil palm plantations and burning changes microbial communities in different ways.

The Burnt land-use was distinct from other land-use types because of high relative abundance of primarily Gram-negative biomarkers namely *18:1n7, 16:1n9* and *cyc17:0*. This is possibly due to higher water table in the Burnt land-

use because of drain blocking (Table 1). Gram-negative relative abundance positively correlated with volumetric moisture. Similar correlations were also reported by Dhandapani et al. (2019b), where primary peat forest with above surface water table, was dominated by Gram-negative bacteria.

Consistent and Coherent Changes with Depth Irrespective of the Land Use

The observed change in community structure with depth, may be influenced by several factors such as water table level, availability of oxygen and other nutrients, quality of C, and other energy constraints, along the peat depth gradient (Andersen et al. 2013). This change with depth in Forest land-use was anticipated, as the surface microbial communities in North Selangor peat forest were dependent on C leached from leaf litter (Yule 2010), and the microbial communities were expected to change with changes in C source and quality with depth (Artz et al. 2007). However, similar changes with depth were also observed in other land-use types, including Burnt, Mature and Young Oil Palm land-use types, all of which lack a leaf litter layer in the surface, indicating that there are more factors than altered litter input driving this change in community structure.

Research conducted using genetic analyses in Northern peatlands show vertical stratification of microbes, with typically greatest microbial diversity and abundance in the intermediate depth because of the co-existence of oxic and anoxic layers, and reduction in diversity with increasing depth thereafter (Andersen et al. 2013). It is not known whether tropical peatlands follow similar pattern and it was beyond the scope of this study. However, Too et al. (2018) also found decrease in diversity in 45 and 90 cm depths compared to the surface layers in NSPSF, and *16S rRNA* sequencing by Jackson et al. (2009) found that microbial diversity decreased with depth (down to 50 cm) in North Selangor peatlands, suggesting vertical stratification at least in surface layers. It is interesting that the relative abundance and the community dominance of the Gram-positive bacteria were also diminished with depth. There is a need for further research to understand if the loss of Gram-positive diversity with depth (Jackson et al. 2009) affected their community dominance over other microbial community groups.

Increase in non-specific fatty acids, with decrease in Gram-positive bacteria influenced the change with depth below the surface, however further species level studies are required to further understand the dynamics of this change. The fungal relative abundances were low throughout the depth, contrary to the finding in northern hemisphere where fungi dominate the surface aerobic layers in natural forest ecosystems (Bardgett 2005; Winsborough and Basiliko 2010). Gram-negative bacteria either remained unchanged or increased slightly with depth. This is likely be due to the increase in C:N ratio with depth (Supplementary Information 3; Tonks et al. 2017), which favours Gram-negative bacteria (Bossio and Scow 1998; Liu et al. 2015). A sudden spike in F:B and G-:G+ ratios at the deepest layers of mature oil palm plantations may be due to a sudden and extreme change in soil properties due to the presence of a mineral layer at the greatest depths, as the depth of the peat cores in mature oil palm were very shallow compared to the other land-use types (Tonks et al. 2017).

This distinct shift in microbial community structure after a metre depth was not directly related to the water table level, as water table levels varied between different land use types during the time of sampling and they were not at a metre depth at any of the studied sites (Table 1; Tonks et al. 2017). Reasons for the shift in microbial communities after about a metre depth are therefore unclear. However, it is possible that the old peat deposits below a metre depth at the catotelm (Morris et al. 2011) are dense and recalcitrant (Tonks et al. 2017; Cooper et al. 2019), causing changes in microbial communities through changes in bulk density and hence blocking water movement (Andersen et al. 2013). There is a possibility that archaeal communities also occur at that depth range in North Selangor peatlands (Too et al. 2018), however the impact of their occurrence on other microbial communities are unknown. Jackson et al. (2009) did not find methanogens in the first 50 cm from the peat surface in North Selangor peatlands, however Too et al. (2018) found increased richness of archaea at 90 cm depth in the same North Selangor peatlands. Similarly, methanogens were found to be abundant at 30–40 cm below the water table in Northern Peatlands (Galand et al. 2002; Lin et al. 2014). Tropical peatlands may exhibit similar pattern for the occurrence of methanogens, which is at approximately 1 m depth in North Selangor peatlands when accounting for long-term water table draw down levels in most of our studied sites (Tonks et al. 2017). As PLFA analyses do not detect methanogenic archaeal communities, due to their ether link phospholipid cell membranes (Gattinger et al. 2003), rather than ester linked fatty acids found in other bacteria and fungi, there is a knowledge gap in addressing the shift, the occurrence of methanogenic communities and their relationship. Future studies may use molecular genetic techniques to characterise archaeal communities and also identify the shift at a metre depth.

Bacterial Dominance in Community Structure

The generally observed pattern in previous studies in different ecosystems are for bacterial dominance in heavily disturbed or intensive agricultural sites, while the sites with lesser disturbance, more acidic conditions and greater input of complex organic matters in natural habitats tend to favour fungal dominance (Bossuyt et al. 2001; Bardgett 2005; Fierer et al. 2009). This did not hold true for the Malaysian tropical peatlands, as

all of the studied land-uses were clearly dominated by bacterial groups across all depths. This leads to the question of whether the removal of bigger dipterocarp trees by historical selective logging (Saiful and Latiff 2014) affected ectomycorrhizal fungal communities (Lee et al. 2010; Brearley 2012), However Too et al. (2018) studied three species of trees including one from Dipterocarpaceae in NSPSF and found that tree species did not significantly influence any changes in microbial community composition in surface and along the peat depth. Additionally, it has also been found that the surface peat layers of primary peat swamp forests in a different location within Peninsular Malaysia that had no known history of disturbance, was also overwhelmingly dominated by bacteria over fungi in the community structure (Dhandapani et al. 2019b). So it is highly plausible that tropical peatlands are inherently dominated by bacteria.

All the surface layers, except for the Burnt land-use were dominated by Gram-positive bacteria, but soon were replaced by Gram-negative bacteria, with increasing depth, possibly due to changes in moisture conditions and C:N ratios (Fig. 7b; Supplementary information 3). Jackson et al. (2009) also found similar results with Acidobacteria, a phylum belonging to Gram-negative bacterial group, being the single biggest group from surface to 50 cm, with considerable increase in their proportion at 50 cm, the deepest layer in their study. All the studied peat sites had higher nitrogen content in the surface (supplementary information 3), which favoured Gram-positive bacteria (Fig. 6c) over Gram-negative bacteria. This is in agreement with study by Liu et al. (2015), who found that Gram-negative PLFA abundance decreased with the addition of nitrogen irrespective of the land use type in humid tropical forests in Southern China. A similar trend of increase in Gram-positive bacteria over Gram-negative bacteria with decreased C:N was also observed in other tropical soils (Smith et al. 2014).

The dominance of Gram-positive bacteria over Gram-negative bacteria on Forest and plantation peat surface layers, is in contradiction with what was observed in Panamanian peatlands, where Acidobacteria and Proteobacteria, both Gram-negatives, together constituted 68–80% of bacterial communities on surface of peat forest (Troxler et al. 2012). Similarly, surface layers of pristine peat forests in Terengganu were also found to be dominated by Gram-negative bacteria (Dhandapani et al. 2019b). This contrast may possibly be due to the difference in water table level between primary and secondary peat swamp forests (Dhandapani et al. 2019b), as reflected by Gram-negative dominance in surface peat layers of Burnt sites, that were submerged due to drain blocking (Table 1). This theory is supported by contrasting significant correlations exhibited by Gram-negative and Gram-positive microbial groups to moisture level (Fig. 6f, d). Jackson et al. (2009) found extracellular enzyme activities were greatest at the peat surface and decreased with depth at the North Selangor peat swamp forest, which corresponds with relative abundance of Gram-positive bacteria. This suggests that Gram-positive bacteria is the most dominant group involved in extracellular enzyme activity and C mineralization in this tropical peatland. Actinomycetes were found to be more active at higher pH (Charousová et al. 2017). Higher pH is also known to support higher bacterial diversity in oil palm plantations on mineral soil (Tripathi et al. 2012; Wood et al. 2017). Even though pH was higher in the plantations than the forest, it was still under pH 4 (Tonks et al. 2017) and did not affect the relative abundance of actinomycetes. This slight increase in pH with establishment of plantations in peatlands, may not be high enough to support increased bacterial diversity, as seen on plantations in mineral soil. Though this study does not indicate bacterial diversity, the ratio of fungi to bacteria were generally higher in Mature Oil Palm plantations (Fig. 4), showing that the slight increase in pH did not favour the bacterial community in peatlands. Actinomycetes, despite being Gram-positive bacteria, are more similar to fungi in structure and functions, while being much smaller in size to that of fungi (Barka et al. 2016). It is interesting to see that they exhibit similar trends throughout the depth at different land uses, and also both exhibiting negative correlations to moisture (Figs. 6b and h). These data are in agreement with those of Bossio and Scow (1998) and likewise their conclusion that fungal PLFA markers were less abundant in submerged systems.

Is there Greater Nutritional Stress under Forest?

Microbes transform some fatty acids in their cell membranes to more stable compounds as an adaptation to maintain functional living cell membranes during stressful conditions (Kaur et al. 2005). The fatty acids *cyc 17:0* and *cyc 19:0* are formed by transmethylation of *16:1n7* and *18:1n7* (Kaur et al. 2005). This transmethylation was found to be a modification in cell membranes to minimize cellular degradation and membrane losses at the times of stressful conditions (Kaur et al. 2005). The higher ratio between *cyc17:0 + cyc19:0* and their precursors *16:1n7 + 18:1n7* is used as a stress indicator for starvation and nutritional stress, due to low C, high acidity and low oxygen (Kieft et al. 1994; Bossio and Scow 1998; Wilkinson et al. 2002; Liu et al. 2015). The ratio was found to decrease with high substrate availability (Bossio and Scow 1998). The ratio did not significantly differ between sites, however decreased with depth, implying decrease in stress level with depth (Fig. 5a). This decrease in ratio was related to an increase in C:N ratio with depth (Fig. 7e). Increase in C availability increases mono-unsaturated fatty acids, resulting in a decrease of cyc:pre ratios. Several studies suggest that an increase in the ratio of saturated fatty acids to mono-unsaturated fatty acids is an indicator of nutritional stress (Zelles et al. 1992; Kieft et al. 1994; Bossio and Scow 1998; Moore-

Kucera and Dick 2008). This ratio was highest at the Forest and lowest at the Burnt land-use. The ratio thus suggests Forest land-use to be nutritionally stressed. It is known that burning makes more nutrients available in peatlands (Turetsky et al. 2015) while the natural tropical peatland habitats are naturally nutrient poor (Sjögersten et al. 2011). However, it should be noted that even though these ratios are widely used as indicators of stress (Willers et al. 2015), the change in ratio could simply be because of the shifts in microbial community structure (Frostegard et al. 2011). Irrespective, the combination of all the results show that Burnt land-use had the most different microbial communities from the Forest, among the studied land-uses of anthropogenic disturbances.

Conclusions

This study has demonstrated that bacteria dominate the microbial community structure in tropical peatlands irrespective of the land use and depth, however the microbial phenotypic structure also strongly responds to anthropogenic disturbances and vary with peat depth irrespective of the land-use. Burnt peatland and oil palm plantations were found to differently affect the soil microbial communities. Though the fatty acid composition varied the most from Forest in both Mature Oil Palm plantations and Burnt peatlands, changes in overall phenotypic structure of microbial communities were relatively less in Mature Oil Palm compared to Burnt land-use, which had the most different microbial phenotypic structure to that of the Forest. However, these changes in microbial phenotypic community structure were found to be closely associated with moisture and water table levels than other characteristics of the land-use. Fatty acid ratios used as ecological indicators also point towards Burnt peatland to have the most different microbial communities to that of peat Forest. Future research should focus on understanding species-level alterations and functional roles of each microbial species with land-use change and fire, and environmental controls on these microbial communities, as the changes in functions of microbial communities in these carbon rich ecosystems could have consequences well beyond the border of their respective ecosystems. Considering that microbes play a major role in the formation and sustenance of peatlands, as well as the amount and species of GHG emissions, incorporating these understandings to microbial processes, their environmental interactions and functional roles into wider peat management plan is critical for improving peat restoration efforts in South East Asia.

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